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Species composition of anamorphic fungi on medicinal plants in Azerbaijan

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This work was devoted to the study of mycobiota of the medicinal plants included in the flora of Azerbaijan, and pathologies caused by the anamorphic fungi. The results demonstrated that the anamorphic mycobiota of the medicinal plants (57 species) in the conditions of Azerbaijan is abundant and various and the mycobiota of the medicinal plants of Azerbaijan consists of 110 species of anamorphic fungi belonging to 22 genera. The most representative genus consisting of 10 and more species in each type are: *Septoria* – 14, *Penicillium* – 12, *Ascochyta* – 11, *Fusarium* – 10, *Aspergillus* – 10, other types are represented by 1-8 species. It was also demonstrated that the discovered fungi include the species which cause the different diseases (ascochitosis, septorioz, necrosis, spotting, rubigo, powdery mildew, botritis disease, wilting, phomosis etc.), and the frequency of occurrence of the activators of these pathologies varies greatly within 0.001-17.6%. Among the fungi found on medicinal plants, there are few toxigenic species. The presence of toxigenic fungi and their mycotoxins on the plants used for medicinal purposes is unfavorable and justifies the development of missing or non-existing approaches regulating both the presence of fungi and the quantity of their mycotoxins

Keywords: *Medicinal plants, mycobiota, anamorphic fungi, fungal diseases, frequency of occurrence*

INTRODUCTION

It is commonly known that currently the plant kingdom includes about 0.5 mln species. About 16% of these plants are medicinal, and many of them are used in the folk medicine over a long time (Chen et al., 2016). Idea does not correspond to the cited literature, also type of citation is unusual. However, many of these plants did not pass the screening for detection of medicinal properties, with the assumption that about 4/5 of global population administer the plant preparations and demand for such preparations increases every year (Ivanise et al., 2018; Noudèkè et al., 2017). Regardless of this fact the reserves of plants used for receiving the same preparations are limited gradually (Yuan et al., 2016). On the one hand, it is associated with the biological diversity depletion, and on the other hand – with intense, irrational and insufficiently controlled preparation of raw materials. It should be also noted that some medicinal plants are used for other purposes (feeding, food and technical) that plays a certain role in limiting the reserves of these plants. Together with it, it is necessary to consider the diseases of these plants caused by the fungi, even regardless of that many medicinal plants have the antifungal and antibacterial action (Safarova et al., 2018; Sarkhosh et al., 2018). As a result, all this negatively affects the resources of medicinal plants. That is why studying the regularity of the occurrence and distribution of the phytopathogenic properties of fungi in relation to the medicinal plants is also important in this context.

The natural vegetation of the Republic of Azerbaijan differs by great variety and reflects not only the impact of the set of modern natural and historical conditions and human but also the complex and long-term historical path of formation backgrounded by the change of geological period. Almost all large types of vegetation found on the

globe are represented at comparatively small territory. Currently, about 4700 species belong to the flora of Azerbaijan (Mehdiyeva, 2011). Together with the species of plants widely found in Caucasus and other countries the flora of Azerbaijan has the significant number of species specific only for Azerbaijan (Hirkan figs - *Ficus hyrcana* A.Grossh. T.). Special attention shall be paid to the relict plants (iron wood – *Parrotia persica* (DC.) C.A.Mey., silk tree – *Albizia julibrissin* Durazz., chestnut-leafed oak – *Quercus castaneifolia* C.A.Mey., boxwood – *Buxus* L., etc.) which appeared about 70 million years ago.

It should be noted that the flora includes many species considered as useful among which the special place is occupied by the medicinal plants (about 1500 species). The medicinal plants include all the life forms (grasses, bushes and trees), both wild-growing and cultivated species. In addition, the medicinal plants of Azerbaijan also include the endemic and relic species (pomegranate – *Punica* L. and fig tree – *Ficus carica L.*). Some medicinal plants belonging to the flora of Azerbaijan are widely used for feeding (*Trifolium pratense* L., *Zea mays* L.), food (*Cucurbita* L., *Zea mays* L., *Solanum L.*) and technical (*Fagus orientalis* Lipsky, *Pinus* L., *Populus* L. etc.) purposes (Mehdiyeva, 2011).

Regardless of that the flora consists of many medicinal plants they are studied insufficiently, especially in the mycological context. In this relation, this work was aimed at studying the mycobiota of the medicinal plants belonging to the flora of Azerbaijan and pathologies caused by the anamorphic fungi.

The selection of anamorphic fungi is stipulated by that, first, the fungal pathologies are more common among the plants compared to bacteria and, second, the most of phytopathogenic fungi belong namely to these species (Doehlemann et al., 2017).

MATERIALS AND METHODS

The medicinal plants of Azerbaijan were used as the material for this study. The samples of medicinal plants were collected during the 10th expedition (2010-2018) at the different territories of Azerbaijan (Great Caucasus, Talysh mountains, Kura-Araks lowland, part of the territory of Lesser Caucasus not occupied by Armenia).

In all cases, rice agar (RA), potato agar (PA) were used from the wort agar (WA), agar medium Czapek (AMC) (Handbook of Mycological Methods, 2006). It was also used during the hiding of workers' cultures from these nurturing environments.

When identifying fungi taken into a pure culture from the abovementioned nutrient media, determinants based on cultural-morphological and physiological characters were used (Sutton et al., 2001; Klich, 2002; Kirk et al., 2008). For the taxonomy and the name of the fungus, the data indicated on the official website of IMA (Crous et al., 2004; Robert et al., 2005; Vincent et al., 2013). The determination of pathology caused by fungi was also carried out as in Horst (Horst, 2013).

The frequency of occurrence (P) of the species (diseases) was evaluated (%) using the following formula: $P=(n/N)$ x 100, where n - is a number of samples on which this species of fungi (type of disease) are detected, N - is a total number of samples.

RESULTS AND DISCUSSION

The results demonstrated that the anamorphic mycobiota of the medicinal plants in the conditions of Azerbaijan is abundant and various which is confirmed by the data specified in table 1. As it can be seen, the mycobiota of the medicinal plants of Azerbaijan consists of 110 species of anamorphic fungi belonging to 22 genera. Some of the representative genera consist 10 and more species: *Septoria* – 14, *Penicillium* – 12, *Ascochyta* – 11, *Aspergillus* – 10; other genera are represented by 1-8 species. Therefore, above mentioned 4 genera make up about 42.7% of detected mycobiota and include 47 species.

The selectivity in the distribution of micromycetes by 57 species of host plants also were characterized in different manner and the largest number of micromycetes (more than 10 species) was detected mainly on the plants of three genera: *Solanum* (11 species of anamorphic fungi), *Trifolium* (15 species) and *Zea* (10 species). 1-8 species of anamorphic fungi were detected on other 54 species of plants.

As it is shown in table 1 numerous micromycetes detected on plant genera. Associations between fungi and plant most probably could be explained by ecological conditions of different study territories within Azerbaijan.

It is known that many fungi cause spotting in plants, the causative agent of which are representatives of different genera. The results of our studies showed that most of fungi detected on medicinal plants also cause spotting, and the frequency of occurrence of which relative to the total number of plants studied is average 17.6%.

Such pathologies are specific for *Septoria* fungi in relation to which during the study it was established that the species of this genus cause the spotting of leaves in the plants of 15 genera. The detected fungi include *S.astericola* Ellis et Everh., *Septoria alliorum* West., *S. carotae* Nagorny., *S.leucanthemi* Sacc et Speg, *S. lucopersici* Speg and *S.petroselini* (Lib) Desm, which are common in the territory of Azerbaijan, and they are characterized by a relatively high frequency of occurrence (4.0-6.0%), that such species as *S. iridis* C.Massal., *S.primulae* Buckn., *S.cacaliae-aconitifoliae* Ziling., *S.senecionis* Westend., *S.violae* Rabenh., *S. flagellifera* Ell. et Ev., *S.glycines* T.Hemmi. and *S.valerianae* Sacc.et Fautrey (0.001-1.0%). The range of feeding plants for the most of detected *Septoria* species is limited to one genus of the host plant, except for *S.violae* which were detected on two species of *Viola* (*V.arvensis* Murr. and *V.odorata* L.).

Table 1. Distribution of fungal genera and species on host plants.

№	Fungal genera	Number of species	Genera of host plants
1	*Acremonium*	1	*Rosa*
2	*Alternaria*	8	*Agropyrum, Brassica, Calendula, Datura, Heliantus, Iris, Malva, Nicotiana, Senecio, Punica, Solanum, Trifolium, Verbascum, Zea*
3	*Ascochyta*	11	*Aconitum, Iris, Primulla, Punica, Senecio, Trifolium,Valeriana, Verbascum, Zea*
4	*Aspergillus*	10	*Agropyrum, Calendula, Datura, Heliantus, Malva, Nicotiana, Senecio, Punica, Solanum, Trifolium, Verbascum, Zea*
5	*Biopolaris*	1	*Iris*
6	*Botrytis*	2	*Iris, Trifolium, Zea,*
7	*Cercespora*	2	*Foeniculum, Viola*
8	*Cladosporium*	3	*Achillea, Carum, Ficus, Datura, Trifolium, Zea*
9	*Colletotrichum*	8	*Aloe, Cucurbita, Euphorbi, Ficus, Pimpinella, Nepeta, Viola, Zea*
10	*Fusarium*	10	*Artemisia, Asparagus, Malva, Magnolia, Petroselinum, Satureja, Solanum, Trifolium, Tulipa, Zea*
11	*Gliocladium*	1	*Tulipa*
12	*Marssonina*	1	*Rosa*
13	*Penicillium*	12	*Astrodaucus, Datura, Dorema, Ficus, Leucanthemum, Ocimum, Phlomis, Punica, Satureja, Trifolium, Tripleurospermum, Tulipa, Zea, Zosima*
14	*Phoma*	8	*Anethum, Artemisia, Brassica, Carum, Chenopodium, Daucus, Ficus, Medicago, Paeonia Stachys, Valerian, Verbascum, Zea*
15	*Phomopsis*	4	*Sambucus, Solanum*
16	*Ramularia*	5	*Galega, Gentiana, Iris, Magnolia,Viola*
17	*Rhizoctonia*	1	*Zea*
18	*Sclerotium*	2	*Laurus, Leucanthemum, Teucrium*
19	*Septoria*	14	*Carum, Daucus, Ficus, Heracleum, Iris, Laurus, Mentha, Primulla, Rhamnus, Senecio, Solanum, Tanacetum, Valeriana, Viola*
20	*Stemphylium*	3	*Achillea, Ficus, Punica, Senecio, Leucanthemum*
21	*Trichothecium*	1	*Anthemis, Rosa*
22	*Verticillium*	2	*Teucrium, Trifolium, Zea*

One of the genus characterized by the rich species diversity is *Ascochyta*. They also affect the different (vegetative and generative) organs of medicinal plants and cause the spotting (or disease- ascochytosis). The detected species (*A. anethicola* Sacc., *Asc. iridis* Oudem., *A. malvicola* Sacc., *A. primulae* Trail., *A. pseudopinodella* Bond.-Mont et Wassil., *Asc. pinodes* Jones. and *Asc. berberidina* Sacc.) are characterized by narrow substrate specifics, i.e. the most of detected species are stenotrophic, though the fungi of this type include also the conditionally stenotrophic species (*Asc. allii-cepae* Punith. and *Asc. phaseolorum* Sacc.) and eutrophic (*Asc.cucumeris* Faurtr. et Roum. and *Asc.doronici* Allesch.).

The fungi of the genus *Alternaria* (*A. alternata* (Fr.: Fr) Keissl., *A.calendulae* Ondfej, *A.cucurnerina* (Ell. et Ev.) Elliot, *A.iridicola* (Ellis et Everh.) J.A.Elliott, *A.longipes* (Ellis et Everh.) E.W.Mason., *A.helianthi* (Hansf) Tubaki et Nishih. and *A. solani* Sorauer.*), Ramularia* (*R.geranii* Fuckel., *R.lactea*(Desm)Sacc., *R. macrospora* Fresen and *R.galegae* Sacc.), *Cladosporium (C.cladosoriodes* (Fresen.) G.A. de Vries, *C.iridis* (Fautrey et Roum.) G.A.deVries and *C.herbarum* (Pers.) Link.), *Colletotrichum* (C. capsici (Syd) Buti et Bisby., *C.circians* (Berk.) Voglino, *C. gloeosporioides* Penz.) Penz. et Sacc., *C.langenarium* (Pass) Ell et Halst., *C.malvarum* (A.Braun et Casp.) Southw, *C. nigrum* Ell. et Halst. *C. orbiculare* (Berk. et Mont) Arx., *C. phomoides* (Sacc)Chest.) also cause the spotting in the medicinal plants. The results demonstrated that the occurrence of these species of fungi is within 0.001-3.2%.

It was shown that only one species of the genus *Botrytis (B. cinerea* Pers) is recoded on the medicinal plants. Despite this, this fungus is one of the most widespread not only throughout Azerbaijan but also in the world (Safaraliyeva, 2019), *B. cinerea* is a significant necrotrophic plant pathogen causing devastating diseases on more than 500 plant species, especially on fresh fruits and vegetables, resulting in the economic losses ranging from $10 billion to $100 billion worldwide (Hua et al., 2018). As a result of our studies, it was shown that the frequency of occurrence of this fungus in Azerbaijan is 4.2%. Affecting the leaves, stalks, flower buds and flowers of the plants they cause the botrytis disease.

According to the table, the *Penicillium* possess rich species diversity, and 12 species (*P. chrysogenum* Thorn., *P. citrinum* Thom., *P. cyclopium* Westl., *P. expansum* Link., *P. funiculosum* Thom., *P.janthinellum* Biourge., *P. lanosum* Westling., *P. olivaseum* Wehmer., *P. puberulum* Bain., *P. rubrum* Stoll. *P. purpurogenum* Stoll. and *P. rubrum* Stoll.) form the mycobiota of the medicinal plants. The certain pathology associated with these fungi are not studied yet (except for the mold formation) but their presence on the medicinal plants weakens them and creates the favorable conditions for other phytopathogens. In addition, the species of this type are able also to synthesize the toxins together with other fungi.

During the study some species causing wilting was detected on the medicinal plants, such as *Verticillium albo-artrum* Reinke and *V.dahliae* Klebahn., fusariosis by F*uzarium moniliforme* J.Sheld., *F.oxysporum* Schlechtend., *F.sambucinum* Fuckel. *F.semitecthum* Berk., *F.solani*(Mart)Sacc., phomosis by *Phoma anethi* (Pers) Sacc., *Ph. cepae* Verwold et Du Plessis, *Ph. exigua* Desm., *Ph. minutella* Sacc. et. Penz., *Ph.rostrupii* Sacc., *Ph.siliguarum* Sacc et Rourn, *Ph. solanicola Prillet.* Delacr. and *Ph.subvelata* Sacc., etc. The occurrence of the activators of these diseases varies within 0.01-2.1%.

It should be noted that the fungi causing one or another pathology not only weaken the biological activity of the plants but also fertilize them by their metabolites which include also the toxic substances. The presence of fungi and their mycotoxins on the plants used for medical purposes is unfavorable and even hazardous because the results of their impact on the health of humans are characterized negatively (Anater et al., 2016). In addition, currently many countries have the normative documents regulating the mycological safety in relation to the use of such materials which do not consider all the details of the actions of the above mentioned groups of fungi that justifies the development of missing or non-existing approaches regulating both the presence of fungi and the quantity of their mycotoxins.

CONCLUSION

Thus, in the studies it was found that the anamorphic mycobiota of medicinal plants in Azerbaijan is abundant and diverse. Mycobiota of medicinal plants of Azerbaijan consists of 110 species of mushrooms of the 22nd genus. Despite the fact that the conducted studies concerned a small part of medicinal plants, the results obtained to some extent expanded the information on anamorphic fungi associated with medicinal plants of Azerbaijan, and many species were first discovered on medicinal plants in Azerbaijan.

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Azərbaycanda dərman bitkilərində anamorf göbələklərin növ tərkibi

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Təqdim olunan işin məqsədi Azərbaycan florasına daxil olan dərman bitkilərinin(57 növ) mikobiotasının və anamorf göbələklərin onlarda törətdikləri patologiyaların tədqiqinə həsr edilibdir. Göstərilmişdir ki, Azərbaycanın dərman bitkilərinin mikobiotası zəngin və geniş müxtəlifliyə malikdir, belə ki, Azərbaycanın dərman bitkilərinin anamorf mikobiotasına 110 növə aid 22 cins daxildir. 10 və daha cöx növlə təmsil olunan cinslər: *Septoria* -14 növ, *Penicillium* - 12, *Ascochyta* – 11, *Aspergillus* – 10. Qalan cinsəlr isə 1-8 növlə təmsil olunuralr. Müəyyən edilmişdir ki, qeydə alınan göbələklərin əksəriyyəti dərman bitkilərində müxtəlif xəstəliklər (ləkəlilik, boz çürümə, fusarioz, solma və s.) törədir və həmin patologiyaların rastgəlmə tezliyi güclü şəkildə variasiya edir və 0,001%-17,5% arasında dəyişir. Dərman bitkilərində qeydə alınan bitkilər arasında bəzi toksigen göbələklərə də rast gəlinir. Tibbi məqsədlər üçün istifadə edilən bitkilərdə toksigen göbələklərin və onların mikotoksinlərinin olması əlverişli deyil, bu səbəbdən də göbələklərin həm özlərinin, həm də əmələ gətirdikləri mikotoksinlərin miqdarını tənzimləyən yanaşmaların işlənib hazırlanmasını əsaslandırır.

Açar sözlər: *Dərman bitkiləri, mikobiota, anamorf göbələklər, göbələk xəstəlikləri, rastgəlmə tezliyi*

Видовой состав анаморфных грибов на лекарственных растениях в Азербайджане

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Целью представленной работы явилось изучение микобиоты лекарственных растений, входящих во флору Азербайджана, и патологий, вызванных анаморфными грибами. Показано, что анаморфная микобиота лекарственных растений Азербайджана обильна и разнообразна.Так, в Азербайджане насчитываются относящиеся 22 родам 110 видов анаморфных грибов лекарственных растений. Наиболее полно представленными родами, которые насчитывают по 10 и более видов в каждом роде, являются: *Septoria* - 14 видов, *Penicillium* – 12 видов, *Ascochyta* – 11 и *Aspergillus* – 10 видов. Остальные роды представлены 1-8 видами. Выявлено, что большинство грибов, обнаруженных на лекарственных растениях, вызывают различные болезни (пятнистость, серая гниль, фузариоз, увядание и др.), и частота встречаемости возбудителей этих патологий сильно варьирует и находится в пределах 0,001-17,6%. Среди грибов, встречающихся на лекарственных растениях, существует несколько токсигенных видов. Наличие токсигенных грибов и их микотоксинов на растениях, используемых в медицинских целях, является неблагоприятным и оправдывает разработку подходов, регулирующих как наличие грибов, так и количество их микотоксинов.

Ключевые слова: *Лекарственные растения, микобиота, анаморфные грибы, грибные болезни, частота встречаемости*